Reviewer 1:

We thank the reviewer for the comments that have improved the understanding of the manuscript. In particular, the choice of the right terminology made the message much clearer.

Comment:

However, there are discrepancies between the expectations the title stirs by its wording ("fungal biomass"), the aim formulated in the introduction (L128-L130), the hypotheses (L146-152) and the methods. Wording and presentation of goals of the manuscript should be unified and unique aims/results be highlighted: 1) please choose a term that can be derived from the methods and use it uniformly, e.g., "EMF biomass production", because the distinction from similar terms ("fungal biomass") throughout the manuscript is unclear.

Reply:

It is indeed a fair point. The main goal of the paper was to analyse EMF biomass production, so we used the meshbag methods which is well known for selecting EMF from other EMF guilds. However, we lack non-mycorrhizal controls (as it was pointed out in one of your comments below) so we could not confirm the assumption that our measured and predicted biomass was entirely EMF. Therefore, the term *fungal* instead of EMF was used.

However, as it is discussed in the manuscript it is very likely that **most of** the fungal growth registered in the current study is of EMF origin. Therefore, we have decided to use the term **EMF production** instead of fungal production. We have changed the terminology accordingly in the manuscript.

Additionally, we added some motivation for the use of this terminology in the materials and methods:

The fungal cell membrane compound ergosterol, a proxy for fungal biomass, was extracted and measured from 5 g of the pooled samples as per Bahr et al. (2013) using high-pressure liquid chromatography (auto sampler L2130 with UV detector L2400 by Hitachi, Japan). ***It was assumed that after incubation in the soil the meshbags contents were dominated by EMF as it has been shown by metabarcoding (Almeida et al., 2018; Rosenstock et al., 2016; Berner et al., 2012; Wallander et al. 2010; Hedh et al. 2008) and isotopic studies (Wallander et al., 2001). Therefore, the fungal biomass collected was expected to be of EMF origin.***

In the discussion we have added a new section disusing this further.

See one of the comments below that talks about the lack of non-mycorrhizal controls..

Comment:

2) the hypotheses are unconnected bullet-points, which is making them very generic; please integrate them into the last part of the introduction together with the reasoning behind them, especially as a body of prior studies followed similar questions.

Reply:

The hypotheses have been incorporated into the last part of the introduction´s body:

In this study, we aimed to improve our understanding of EMF production and turnover in natural soils by testing how fungal production collected from ingrowth meshbags is affected when P is limiting tree growth. In the forest described by Almeida et al. (2019) we estimated EMF production and turnover using the mathematical model of Ekblad et al. (2016) with Bayesian inferences. ***Our first hypothesis was that P fertilization will decrease EMF biomass production in this P limited forest as a result of the limitation being alleviated.***

In addition, because EMF growth is subsidized by the host, in exchange for N and P, EMF production in the meshbags production should be affected by the nutrients found at the hyphal front. Indeed, EMF biomass in P-poor forests is stimulated around localized patches of the P-rich mineral apatite (Rosenstock et al., 2016; Berner et al., 2012; Hagerberg et al., 2003). Therefore, besides purely sand-filled meshbags, we incubated meshbags amended with apatite or methylene urea (referred as urea throughout the manuscript) in order to simulate soil N and P nutrient patches respectively. ***We expected that the nutrient patches will increase EMF biomass production depending on fertilization. In particular: apatite amendment will increase EMF biomass production in the control plots but not in P***

fertilized plots (second hypothesis) ; and urea amendment will increase EMF biomass production in the P fertilized but not in the control plots (third hypothesis).

Finally, since belowground C allocation follows the three phenological cycles (Endrulat et al., 2016), EMF production is likely to vary with season peaking in autumn (Hagerberg & Wallander, 2002 ;Wallander at al., 2001; Hagenbo et al., 2021), we performed a more extensive incubation scheme and more frequent harvests of bags than in Ekblad et al., (2016). This allowed us to test the model considering the treatments effects (P fertilization and meshbags amendments) and also considering their interactions with seasonality (time of the growing season). ***Therefore, our fourth hypothesis was that EMF biomass production will be higher in autumn than in summer.***

Comment:

3) turnover/seasonality was a central aspect in the study, it should be involved in a hypothesis, too.

Reply:

Seasonality has been included as a hypothesis in the last part of the introduction´s body. See the comment above.

Turnover is an important part of the study however we aimed to measure EMF growth (production) when a forest is P-limited. In that sense turnover rates were not part of the hypothesis but it was a mean to improve our measurements of EMF biomass.

Specific Comments:

- Title: “fungal biomass production” includes all types of fungal lifestyles, including the quantitatively very important group of saprotrophs. However, most of the manuscript uses the

term “EMF production”. Therefore, I suggest to change the wording or explain in detail in the text how EMF production could be a proxy for other fungal lifestyles.

Reply:

We changed to:

Phosphorus regulates ectomycorrhizal fungi biomass production in a Norway spruce forest

- Abstract:

L12: To me, it is not clear, how you arrive at “fungal production” here, when you specify in L10 that you estimated EMF production. This should be clarified throughout the manuscript.

Reply:

Changed to:

Fungal mycelium collected from ingrowth meshbags is commonly used to estimate EMF biomass, but these measurements might not reflect the total fungal production since turnover rates of the hyphae are not considered.

- Introduction:

L86: EMM abbreviation is not explained. Do you mean extrametrical mycelium? (same in L502, L569)

Reply:

Changed

L97: Ekblad et al .2016 do not use the term fungal standing biomass

Reply:

I have change it to EMF standing biomass that is the term they used.

- Methods:

Experiments: was there any kind of control to examine the share of non-EMF fungi in the ingrowth bags, like ingrowth bags in a root-free area of soil, or amplicon sequencing of the EMF that were found in the ingrowth bags? – It would be very helpful to have clear knowledge to which degree this experiment was able to capture the term "EMF biomass production" used throughout the manuscript.

Reply:

Unfortunately, no, we do not have non-mycorrhizal controls in our study.

We have reorganized the last part of the discussion and created a new section where I acknowledge and discuss this weakness of the study:

4.4 Potential non-mycorrhizal growth in the meshbags

It could be also possible that non-mycorrhizal fungi contributed to the fungal growth detected in the current study. The main assumption that the ergosterol in this experiment comes mostly from EMF relies on previous evidence that the meshbag system favors the growth of EMF over non-mycorrhizal fungi (Almeida et al., 2018; Rosenstock et al., 2016; Berner et al., 2012; Wallander et al. 2010; Hedh et al. 2008; Wallander et al., 2001). However, it has been shown that the shorter the time period a meshbag remains underground the higher the proportion of non-mycorrhizal fungi inside the bags (as measured by the proportion of non-mycorrhizal DNA in Hagenbo et al., 2018).

Thus, non-mycorrhizal fungi growth could partially explain the seasonal effect detected as this fungal guild has been reported to respond positively to temperature (Pietikäinen et al., 2005). Unfortunately, the current study lacks non-mycorrhizal biomass controls (ie: fungal biomass from ingrowth bags collected in a trenched root-free area) that can be used to estimate the contribution of non-mycorrhizal fungi. Therefore, we cannot rule out the possibility that part of the ergosterol measured in the bags came from non-mycorrhizal fungi. Even so, the significant negative effect of P fertilization on all the meshbag types suggests

that the decrease in fungal growth might be related to reduction in C allocation by the trees as discussed earlier. Moreover, the effects of the P fertilization and meshbag amendment on fungal growth were higher early in the season which might imply that the seasonal effect seen in the current study is explained mostly by EMF.

It must be noted nevertheless that a potential reduction in belowground C allocation could decrease root activity and possibly root exudates which might reduce labile sugars in the soils affecting saprotrophic fungi as well. Further studies are necessary to evaluate the effect of P limitation on root dynamics and other members of soil microbial communities.

L188-190: drilling a new hole and placing an ingrowth bag in it and re-placing an ingrowth bag by putting it into an existing hole seem to be two different kinds of disturbance. Is there any knowledge on this?

Reply:

In both cases (placing and replacing) there will be severing of hyphal connections either by the soil corer or by removing a bag from the soil. To our knowledge there is no information which one produce the highest disturbance. However, introducing the soil corer that is sharp should make a clean cut in the mycelium. After that a volume of soil which contains some mycelium is removed by the soil corer.

When a bag is removed in order to place a new one, the hyphal connections are also broken and some mycelium is removed inside the meshbag that is taken up.

Since, the meshbag diameter is the same as the soil corer, the broken mycelium has to recolonize the same space in both cases (placing and replacing) . Therefore, the disturbance should be very similar.

Models: I am not versed in modelling and Bayesian inference, wherefore I could not review parts based on this in detail. However, the models seem well thought trough. Unfortunately, there is no explanation of data sources. The statements “the methodology allows us to draw information from publications” (L313) and “Priors for ð□‘*f*k and ð□œ‡k were derived from the literature”

(L322) are too vague. How was this done and which publications where used? Please explicitly state if this is based on data from Hagenbo et al. 2017 (L334) or new estimations. -- All data sources should be clarified and the data made available.

Reply:

Fair enough. We have made it clearer:

In our case the methodology allows us to draw information from previous studies. ***In particular, we used information from a EMF production study in a conifer forest by Hagenbo et al. (2017).***

AND:

Priors for P_k and μ_k were derived from the mean EMF biomass production and turnover for a forest of similar age as the forest in the current study estimated by Hagenbo et al. (2017) after unit conversion. *Both priors were expressed as normal distributions with deviation prudentially estimated as 25% of the mean (please note that this does not mean that the prior was limited within this range, due to the tails of the normal distributions).*

- Results:

L380: do you mean "… apatite, urea, and not amended meshbags"?

Reply:

No, there was a mistake I meant:

for the apatite and the urea amended meshbags (Fig 3).

L389-294 / Fig. 2: Please mention the number of data-points for each boxplot, either by plotting or mentioning n in the figure caption. As I understood the methods section, each box in figure 2 is resembling three samples (one pooled sample per plot), accordingly n = 3. In this case, a boxplot is not very useful in summarizing the data and another type of graph could better be chosen.

Reply:

The boxplots were changed to circles with standard error bars.

The number of samples (n=3) is now specified in the figure legend.

L398-403 / Fig. 3: having connecting lines between the points, on first glance indicates a time series with one starting point, but in fact there are for example several 30 and 60 day starting points and the samples are independent of each other. Therefore, deleting the lines (or making them dotted) would be useful. Additionally, labelling the x-axis with "incubation time of ingrowth bags [days]" would also help for understanding.

Reply:

The lines were made dashed, and the label changed as suggested.

- Discussion:

L481-482: "The fact that more incubation periods and a larger number of bags were used makes the present study more reliable." Please clarify: more reliable than what study? And what is the difference implied by "more … periods" and "larger number"?

Reply:

Indeed, it was not so very clear. That statement was deleted. Only this remains:

Thus, the standing biomass of one given incubation time might not truly reflect the effect of fertilization on EMF growth. The use of the sequential incubation method and the mathematical model allowed us to have a more robust estimate of the effect of P fertilization on the extramatrical mycelium in this forest.

L486: the term "extrametrical mycelium" has not been introduced in the text, so far.

L486-487 "P as a nutrient regulating fungal growth in boreal forest was not reported before": Please exactly define what fungal growth stands for in this case or reword for avoiding conflicts with earlier studies. For example, Aleida et al. 2019 (ref. in this manuscript) already wrote for the same forest: "Soil EMF communities responded more strongly to P than to N" which can be read as P is regulating fungal fungal growth. Not to mention the body of literature therein: "Ekblad et al. (1995) found that the production of extramatrical mycelium peaked under low P conditions. In a field study comparing Norway spruce (Picea albies) forests of varying P status,

Rosenstock et al. (2016) observed greatly enhanced EMF biomass from ingrowth meshbags in the P limited forest […] in P-limited forests, fungal biomass is enhanced by the presence of mineral P sources like apatite (Hagerberg et al., 2003; Berner et al., 2012; Rosenstock et al., 2016). Bahr et al. (2015) reported that apatite addition stimulated ingrowth of EMF in meshbags, especially in N-fertilized plots." (Almeida et al. 2019, https://doi.org/10.1016/j.funeco.2018.05.008). Why aren't those references seen as reports of P as nutrient which is regulating fungal growth?

Reply:

Those studies refer to EMF growth measured in the meshbags after a certain incubation time inside the soil. As it was mentioned in the introduction that standing biomass does not consider turnover of mycelium. In that sense their estimation of EMF growth (production) is different from our study.

Moreover, the "the P-limitation" reported in those studies is different from the P limitation reported here.

For example, in Rosenstock et al. (2016) the forest has P deficiencies due to soil parental material. Hagerberg et al. 2003 & Berner et al. 2012 measure EMF biomass in forest with different "P status" based on foliar P contents. And Ekblad et al. 1995 had a pot experiment where nutrient conditions were adjusted. In our forest P limitation is the result of N deposition and is confirmed by different means: Foliar chemistry, EMF communities and tree growth (in Almeida et al. 2019) and now by EMF growth. In that sense the current study is different and offers more evidence than the studies cited before.

However, this statement is confusing I agree and it contradicts those references in the introduction. Therefore, we have deleted that particular sentence: "*P as a nutrient regulating fungal growth in boreal forest was not reported before"* **. Its deletion does not affect the main message of the discussion.**

L504: It would be worth mentioning that an independent second method measuring the decrease in belowground C allocation due to P is needed for verification in further studies.

Reply:

We have added this:

We propose that the decreased EMF production in the P-fertilized plots in our study is a result of a decrease in belowground C allocation due to reduced tree dependency on EMF for P foraging and acquisition. ***Fine root production and root tip colonization by EMF could be advisable as an independent second method to confirm that the decrease in EMF growth in the P-fertilized plots was an effect of reduced C allocation by the trees.***

Technical corrections:

L21: “EMF and was” – missing word

Reply: Fixed.

L65: missing period

Reply: Fixed.

L118: EFM?

Reply: Fixed

L389-294 / Fig. 2: please label left and right panels (a, b). Remove the cluttering design of R-ggplot’s standard output (grid lines, grey facet-boxes. Y-axis label: what is “per g” referring to? Quartz? Please choose a more exact way to label the y-axis like “µg [Ergosterol] / µg […]”

Reply: The labels have been fixed: Instead of Quartz it says Quartz-only to differentiate from the meshbags amended with apatite or urea. The grids were deleted and the Y legend now says: Ergosterol µg g^{-1}

L398-403 / Fig. 3: Remove the legend and explain in the caption. Remove the cluttering design of R-ggplot’s standard output (grid lines, grey facet-boxes. Y-axis label: what is “per g” referring to? Quartz? Please choose a more exact way to label the y-axis like “µg [Ergosterol] / µg […]”

Reply: The labels have been fixed: Instead of Quartz it says Quartz-only to differentiate from the meshbags amended with apatite or urea. The grids were deleted and the Y legend now says: Ergosterol µg g^{-1}

L604 typo: not --> no

Reply: Fixed